UNIVERSITI PUTRA MALAYSIA

BIOEFFICACY AND INFECTION MECHANISM OF SEVERAL ENTOMOPATHOGENIC FUNGI ON CROCIDOLOMIA BINOTALIS ZELL.

NORZITA HASHIM

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BIOEFFICACY AND INFECTION MECHANISM OF SEVERAL ENTOMOPATHOGENIC FUNGI ON *CROCIDOLOMIA BINOTALIS* ZELL.

By

NORZITA HASHIM

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

April 2000

DEDICATION

To

My parents, Hashim bin Abdullah and Tinam binti Jusoh

My brothers and sisters

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Agricultural Science.

BIOEFFICACY AND INFECTION MECHANISM OF SEVERAL ENTOMOPATHOGENIC FUNGI ON *CROCIDOLOMIA BINOTALIS* ZELLER

By

NORZITA BINTI HASHIM

April 2000

Chairman: Associate Professor Dr. Yusof Ibrahim

Faculty: Agriculture

The role of entomogenous fungi as natural enemies for cruciferous insect pests has recently been explored and several isolates of hypomycetous fungi have been identified. The ability of three entomopathogenic isolates of *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Metarhizium anisopliae* var. *majus* (Metsch.) Sorokin to cause infection against second instar larvae of *Crocidolomia binotalis* Zeller was tested. Dosage-response assays were conducted in an ambient environment of 28 $\pm$ 2°

dark periods.

treatment. Results indicated that all isolates of the entomopathogenic fungi were able to cause mortality on the second instar larvae of *C. binotalis*. Exposure to sprays of a conidial suspension of varying dosages from 20 ml^{-1} to 2 x 10^{7}

larval mortality from 10.0 to 100.0%

conidia ml^{-1}, larval mortality was recorded in excess of 80%. ED$_{50}$

was 1926 conidia ml^{-1} and was significantly much lower than that of *B. bassiana* which

was 5038 conidia ml^{-1} Meanwhile, the ED_{50} for *M. anisopliae* var. *majus* was 20000 conidia ml^{-1}, which was much higher than that for *P. fumosoroseus* and *B. bassiana*. *P. fumosoroseus* also demonstrated the best potency of infection when all its series of concentration took lesser times to reach 50% infectivity (LT_{50}
and *M. anisopliae* var. *majus*.

The infection mechanisms of all isolates of the entomopathogenic fungi were studied histologically by scanning electron microscopy. In general, conidia which landed on the cuticle germinated within 4 to 6 hours after inoculation. The germ tube penetrated the cuticle and the hypha subsequently invaded the tissue. Infected larvae died between 24 - 48 hours of post inoculation. The hyphae broke through the outer cuticle after 2-5 days of post-treatment. Conidiophore were produced and they formed conidia, one or two days later. At this stage, complete invasion of the larvae had occurred.

Results from laboratory bioassays revealed that *P. fumosoroseus* was the most efficacious of the three fungal species tested. As such, the efficacy of *P. fumosoroseus* was tested in the field. Studies were conducted in the farm at Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Four formulations of *P. fumosoroseus* were made, namely conidia in aqueous Tween 80 (0.05%), conidia in kaolin, conidia in palm oil and conidia from cultures in rice flour. Larval mortality was recorded daily until 12 days to determine the cumulative percentage mortality. Mean percent mortality was in excess of 70% for all the treatments except for the untreated control. Conidia in palm oil was able to inflict the highest larval mortality (88.5%) and there was no significant difference

compared with conidia in aqueous Tween 80 which recorded 78.0%. Conidia from rice flour cultures effected 76.0% mortality and this was shown to be significantly less virulent than the conidia in palm oil. A lower larval mortality of 75.0% was observed when sprayed with conidia in kaolin. It is thus concluded that conidia in palm oil have the potential for an effective field application compared with conidia in kaolin and conidia from cultures in rice flour.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian.

BIOEFIKASI DAN MEKANISMA JANGKITAN OLEH BEBERAPA KULAT ENTOMOPATOGEN KE ATAS *CROCIDOLOMIA BINOTALIS* ZELLER

Oleh

NORZITA BINTI HASHIM

April 2000

Pengerusi: Profesor Madya Dr. Yusof Ibrahim.

Fakulti: Pertanian.

Peranan kulat-kulat entomogenus sebagai musuh semulajadi kepada serangga perosak krusifer telah dijelajahi baru-baru ini dan beberapa pencilan kulat-kulat hypomycete telah dikenalpasti. Oleh yang demikian, keupayaan jangkitan tiga pencilan kulat-kulat entomopatogen *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces fumosoroseus* (Wize) Brown and Smith dan *Metarhizium anisopliae* var. *majus* (Metsch.) Sorokin telah diuji ke atas larva instar kedua *Crocidolomia binotalis* Zeller. Asai respon-dos telah dilakukan di dalam keadaan persekitaran terkawal pada suhu 28 ± 2^{o}

bandingan $80 \pm 15\%$ dan dengan keadaan berselang-seli terang dan gelap.

larva dicatat 12 hari selepas pendedahan kepada rawatan semburan konidia. Keputusan menunjukkan kesemua pencilan kulat-kulat entomopatogen berkebolehan menyebabkan kematian ke atas larva instar ke dua *C. binotalis.* Semburan dengan kepekatan konidia

20 ml^{-1} hingga 2×10^{7}

100.0

80%. ED_{50} bagi *P. fumosoroseus* adalah 1926 konidia ml^{-1} iaitu lebih rendah berbanding *B. bassiana* dengan 5038 konidia ml^{-1}. Sementara itu, ED_{50}

majus adalah 20000 konidia ml^{-1},

bassiana. *P. fumosoroseus* juga menunjukkan potensi jangkitan yang terbaik apabila kesemua siri kepekatan mengambil masa yang singkat untuk mencapai 50% jangkitan (LT_{50}) berbanding *B. bassiana* dan *M. anisopliae* var. *majus*.

Mekanisma jangkitan kesemua pencilan kulat entomopatogen telah dikaji secara histologi melalu mikroskop elektron. Secara umum,

bercambah dalam masa 4 - 6 jam selepas inokulasi. Tiub germa menembusi kutikel dan hifa kemudiannya menyerang tisu. Larva yang diserang mati di

selepas penginokulatan. Konidiofor terbentuk dan mengeluarkan konidia, sehari atau dua hari kemudiannya. Pada ketika ini, jangkitan sepenuhnya ke atas larva telah berlaku.

Keputusan daripada bioasai di makmal menunjukkan *P. fumosoroseus* adalah paling berkesan daripada ke tiga-tiga kulat yang diuji.

diuji di ladang. Kajian telah dilakukan di ladang Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

dalam air suling mengandungi Tween 80 (0.05

dalam minyak kelapa sawit dan konidia di dalam tepung beras.

dicatit setiap hari sehingga 12 hari untuk mendapatkan peratus kematian longgokan. Peratus kematian adalah melebihi 70% bagi kesemua rawatan kecuali kawalan.

di dalam minyak kelapa sawit berkebolehan menyebabkan kematian larva paling tinggi

(88.5

mengandungi Tween 80 yang dicatit 78% kematian.

menyebabkan 76.0% kematian dan ini adalah kevirulenan yang lebih rendah dengan bererti daripada konidia di dalam minyak kelapa sawit. Kematian larva yang paling rendah iaitu 75% diperhatikan apabila disembur dengan konidia di dalam kaolin. Dengan ini diputuskan bahawa konidia di dalam minyak kelapa sawit berpotensi untuk kegunaan di ladang berbanding konidia di dalam kaolin dan konidia daripada kultur di dalam tepung beras.

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My utmost gratitude to my parents, brothers and sisters for their boundless support and love. Finally, I wish to express my sincere thanks to all those who have one way or another helped me in making this study a success.

I certify that an examination Committee met on 3 April, 2000 to conduct the final examination of Norzita Hashim on her Master thesis entitled "Bioefficacy and Infection Mechanism of Several Entomopathogenic Fungi on *Crocidolomia Binotalis* Zeller" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committees are as follows:

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Date: 1 8 APR 2000

This thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirement for the degree of Master of Agricultural Science.

KHAMIS AWANG,
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Date: 11 MAY 2000

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NORZITA HASHIM

Date:

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CHAPTER 1

INTRODUCTION

Cruciferous vegetables are economically important throughout the world. In Malaysia, they are grown by small landholders around urban centres, in highlands and in specialised production areas. Often farmers use input-intensive agronomic practices because the sale of these vegetables provide an important source of ready cash income.

In recent years, however, crucifer production has been seriously affected by a steady increase in insect pests. The cabbage-heart caterpillar, *Crocidolomia binotalis* Zell. (Lepidoptera : Pyralidae) is considered as the second most important insect pest of cabbage and other crucifers. Severe damage to cabbage has been reported, especially in the Cameron Highlands (Ooi *et al.*, 1979). The larvae live gregariously feeding at first on the under side of cabbage leaves which may be eventually eaten completely. The growing point is often damaged leading to regeneration of multiple heads. As the larva bores into the cabbage head, it gets protected from contact with insecticides (Ooi, 1988). The larva is extremely mobile and can easily travel two or more meters to reach a host plant.

To control the insect pest, most farmers rely solely on chemical pesticides which are often used indiscriminately. A survey by Ooi and Sudderuddin (1978) showed that a mixture of two or more insecticides were applied in excess of the recommended rates and sprayed as frequent as three times a week. These injudicious practices have resulted in many alarming problems such as development of insecticide resistance, pest resurgence, excessive chemical residues and environmental contamination. Concern about the negative effects of chemical insecticides have led to emphasis on alternative strategies for pest control. Meanwhile, the concept of integrated pest management (IPM) has begun to receive widespread acceptance and this will play an important role in establishing the biological control agents for the future (Jusoh *et al.*, 1982).

Microbial control is defined as part of biological control with the manipulation of pathogens or their by-products to cause the reduction of pest population. The unpredictable results with insect pathogens in field applications and the successful use of broad spectrum pesticides to reduce pest population (Tanada and Kaya, 1993) has caused the development of microbial control agents to lag behind the other methods of insect control, especially the chemical control, though the concept of microbial control originated over a hundred years ago.

Entomogenous fungi have been mention to play a uniquely important role in the history of microbial control of insects. Roberts (1981) defined an entomopathogenic fungus as a "pathogen causing an early death of the host by penetrating and proliferating inside the host, which is killed by being deprived of soluble nutriens in its hemolymph, by invasion or digestion of its tissues, and/ or by the release of toxins from the fungus". Research during the last decade has proved that fungi have a prominent place in insect control because the entomopathogenic fungi constitute the largest group of insect pathogens. Furthermore, some enthomophathogenic fungi are undergoing large-scale field test and a significant advance in development and manufacturing of these agents in the future is expected with recent biotecnological innovation (Khachatourians, 1986).

In Malaysia, indigenous isolates of *Beauveria bassiana* (Bals.) and *Paecilomyces fumosoroseus* (Wize) Brown and Smith have been reported recently in laboratory studies to be effective against the cosmopolitan diamondback moth (DBM), *Plutella xylostella* (L.) (Ibrahim *et al.*, 1998; Hashim *et al.*, 1999). *Metarhizium anisopliae* (Metch.) has also been reported to be infectious to termites (Sajap *et al.*, 1990) and rhinoceros beetles (Sajap, 1991). Thus it is of great interest to know whether these isolates are also efficacious against the other lepidopteran pest of cabbage such as the cabbage-heart caterpillar, *C. binotalis*. Hence, the objectives of this study are:-

1. To determine the bioefficacy of the isolates *B. bassiana*, *P. fumosoroseus* and *M. anisopliae* against *C. binotalis* .
2. To critically examine the mechanism of infection through histopathological examination of infected larvae of *C. binotalis*.
3. To compare the field efficacy of several raw preparations of formulations using the best fungal isolate from the entomopathogenic fungi tested against *C. binotalis*.

CHAPTER 2

LITERATURE REVIEW

2.1 *Crocidolomia binotalis* Zeller

2.1.1 Introduction

Crocidolomia binotalis Zeller or cabbage-heart caterpillar, is a pyralid moth. Zeller described *C. binotalis* in 1852 from a specimen collected in South Africa. Early synonyms of *C. binotalis* were *C. comalis* (Guence) (1854) from central India and *C. incomalis* (Guenee) (1854) from Java.

The moths are almost exclusively found in hot humid highland tropics and constitute a more serious pest problem during the dry season since heavy rains can drown small larvae. The native range of the moth probably embraced both Africa and Asia (Waterhouse and Norris, 1987). *C. binotalis* occurs in extensive areas of west, south and east Africa, throughout the warmer region of Asia, in north-eastern Australia, Carolina Is, Cook Is, Fiji, Indonesia, Marianas, New Caledonia, Niuc, Norfolk Is, Papua New Guinea, The Philippines, Weatern Samoa, Solomon Is and Tonga.This pest is not reported in Europe and the Americas (Waterhouse and Norris, 1987). In Malaysia, it has been placed as second in importance after the DBM. Probably the insect is very much held in check under existing conditions. However, severe damage to cabbage has been reported in the Cameron Highlands (Ooi *et al.*, 1979).

2.1.2 Biology and Ecology

Lifecycle of *C. binotalis* is completed in approximately 28 days, depending on temperature and humidity. The eggs are laid in batches, overlapping each other on the lower surfaces of the leaves (Plate 1). Smooth leaf surface are preferred for oviposition. Most eggs are laid, close to the midrib or the veins on the leaf. The eggs when newly laid are greenish but later turn brown prior to eclosion. They are flat, ovoid and are packed neatly like roof tiles. An egg measures about 0.36mm in width and 0.54mm in length. The number of eggs per batch varies from 8 to 114 per batch (Ooi *et al.*, 1979). Incubation period takes five to fifteen days in the lowland, but depending on temperature (Water and Norris, 1987).

There are five larval instars, and feeding occupies fourteen to forty-two days (Waterhourse and Norris, 1987). First instar larvae are slender. Head and thorax are dark brown and the body is greenish yellow in color (Plate 2). The second instar is green and the head is brown with light pathes (Plate 3). The late larval instars are characterised by whitish longitudinal stripes, three dorsal and one on each lateral side (Plate 4). All the abdominal segments have tubercles. Between the dorsal and lateral stripes are found black round spots. These are the bases of long hairs on the body.

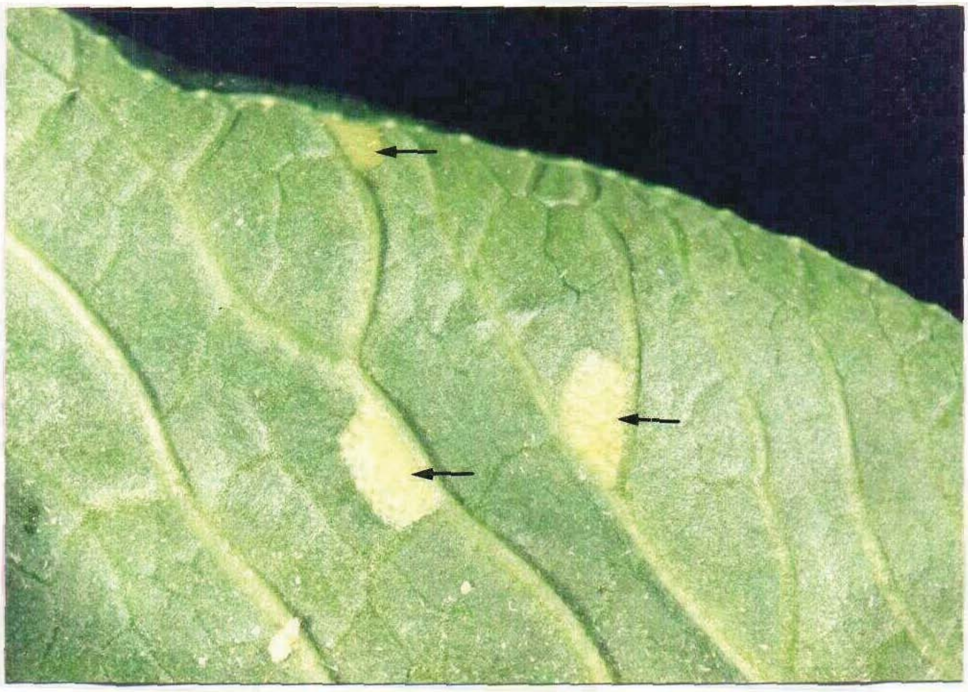

Plate 1 : The eggs of *C. binotalis* are laid in batches on the lower surfaces of the leaf.

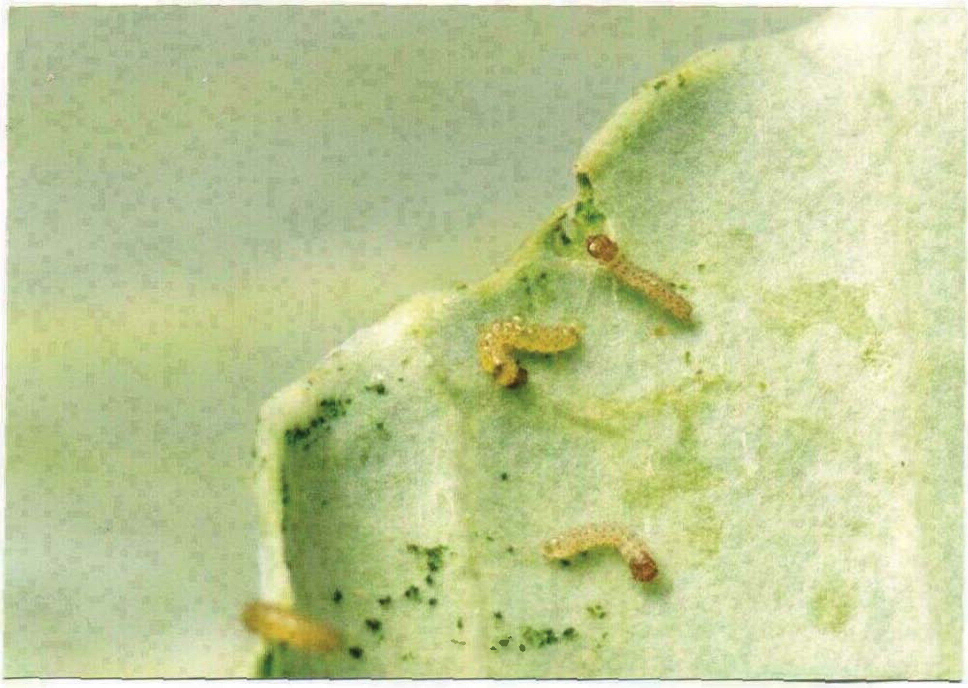

Plate 2 : First instar larvae of *C. binotalis* are slender and the body is greenish yellow in colour (about 2 mm).